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Role of $GABA_A$ receptors in EEG activity and spatial recognition memory in aged APP and PS1 double transgenic mice

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ABSTRACT

Alzheimer's disease (AD) is a leading cause of dementia, with no effective treatment currently available. However, targeting the aging mechanism may improve outcomes and γ-aminobutyric acid (GABA) system alteration could have implications for treatment of cognitive decline in old age. We studied the effects of the GABA system on brain activity in aged APP and PS1 transgenic mice. Low dose (0.1 mg/kg i.p.) $GABA_A$ agonist muscimol and antagonist bicuculline were administered for moderate system activation and inhibition, respectively. EEGs from the hippocampus (Hip) and prefrontal cortex (PFC) were recorded under spontaneous state and during Y-maze performance. Basally, AD mice exhibited increased spontaneous EEG delta (2–4 Hz) and decreased spontaneous EEG alpha (8–12 Hz) activity in the Hip, and decreased Y-maze EEG theta (4–8 Hz) activity in the PFC. Interestingly, $GABA_A$ activation and inhibition in AD mice reduced EEG delta activity and increased EEG theta activity in the PFC, and behaviorally improved spatial recognition memory during Y-maze testing. Decreased spontaneous EEG delta activity was also observed in the PFC. Specifically, $GABA_A$ activation primarily affected low frequency EEG (2–12 Hz) activity in the PFC, whereas inhibition affected EEG activity across many frequencies in the PFC and Hip. These data provide evidence for slower brain activity in AD mice. Importantly, improved spatial memory after $GABA_A$ activation and inhibition may be explained by brain rhythm recovery in certain regions. Our study highlights the potential clinical use of $GABA_A$ drugs to improve cognitive disorders and restore neural network activity in AD.

1. Introduction

Alzheimer's disease (AD) is an important societal health concern. It is the most common cause of dementia in the elderly and afflicts people with increasing age. To date, however, effective treatments that can slow or cure the disease are lacking. Many clinical trials and drug developments in AD have resulted in disappointing outcomes (Abbott and Dolgin, 2016; Anand et al., 2014), suggesting the need for further understanding of the mechanism of AD. As aging is the main risk factor for AD, it has been proposed that targeting the aging mechanism may produce better outcomes (Lucanic et al., 2013).

Normal aging is accompanied by an imbalance in neurotransmitter systems between excitation and inhibition (E/I), including the glutamate and γ-aminobutyric acid (GABA) systems. The aging process can severely impair the GABA system (McQuail et al., 2015; Rozycka and Liguz-Lecznar, 2017), which has implications for the treatment of cognitive decline in old age (Leventhal et al., 2003). For example, older animals show decreased orientation and direction selectivity and increased spontaneous activity in visual cortical neurons, consistent with age-related degeneration of GABAergic inhibition (Schmolesky et al., 2000). In addition, the GABA and GABA type a ($GABA_A$) receptor agonist muscimol can restore the functional selectivity of neurons degenerated with age (Leventhal et al., 2003).

Previous studies on AD have primarily focused on aberrant excitatory neuronal activity (Palop et al., 2007; Palop and Mucke, 2009), such as in glutamate and choline systems, with less attention paid to the GABA system. However, dysfunction of this system is reportedly involved in AD neuropathology, particularly in cognitive deficits (Palop and Mucke, 2016; Verret et al., 2012). Furthermore, GABAergic drugs have been recently applied for cognitive recovery in AD treatment

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(Anand et al., 2014) and adjustment of E/I balance via the GABA system is reported to play a potential role in AD treatment at the early stage (Nava-Mesa et al., 2014; Rissman and Mobley, 2011).

Therefore, in this study, we explored the role of the $GABA_A$ system in brain activity of AD transgenic mice at a late pathological stage. We recorded EEGs from the prefrontal cortex (PFC), hippocampus (Hip), and occipital cortex (Ctx, serving as a control) in mice carrying APPswe and PS1dE9 genes and in their wild-type (WT) littermates under spontaneous state and during Y-maze task performance. Both the $GABA_A$ agonist muscimol and antagonist bicuculline were administered at low doses (0.1 mg/kg for both) due to their possible paradoxical effects on cognition (Pilipenko et al., 2018). Accumulated evidence suggests that 0.1 mg/kg can be considered as a relatively low dose for both drugs. For instance, Pilipenko et al. (2018) defined 0.01 and 0.05 mg/kg as very low doses for muscimol and Castellano et al. (1993) defined 0.5 mg/kg and 0.1 mg/kg as sub-doses for muscimol and bicuculline, respectively. In addition, previous studies have suggested that a bicuculline dose of 0.1 mg/kg is well below the convulsion-inducing dose (Brioni and McGaugh, 1988; Yajima et al., 2000). This study should improve our understanding of the pathophysiology of brain oscillations in AD and of the relationship between network dysfunction and cognitive deterioration.

2. Materials and methods

2.1. Animals

Amyloid precursor protein/presenilin-1 double transgenic male mice (strain name: B6/JNju-Tg (APPswe, PSEN1dE9)/Nju; abbreviated to AD mice) and their WT littermates were purchased from the Nanjing Biomedical Research Institute of Nanjing University (license number SCXK [Su] 2015-0001) (Wang et al., 2015). The transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) directed to central nervous system (CNS) neurons. The mice develop beta-amyloid (Aβ) deposits in the brain by 6–7 months of age. Genotypes were confirmed by polymerase chain reaction (PCR) genotyping at the Nanjing Biomedical Research Institute of Nanjing University using genomic DNA extracted from tail tissue samples.

Mice were individually marked with metallic ear-tags. During the experimental sessions, the mice were housed in groups of 1–3 animals in plastic cages (30 × 18 × 14 cm) under constant temperature (23 ± 1 °C) and stable humidity with a natural light-dark cycle and free access to food and water. All experimental and animal care procedures were carried out in accordance with the guidelines for the National Care and Use of Animals and approved by the National Animal Research Authority. Furthermore, all experiments were approved by the local Committee on Animal Use and Protection.

Surgery was performed on AD and WT mice at 50 weeks of age, at least one week before the first EEG study (please refer to the *Surgery* section). Fig. 1A provides details on the numbers and ages of each experimental group. Data were analyzed from surviving animals during the entire recording period. For EEG analysis, data from individual recording channels were removed because of loss of electrode contacts and EEG artifacts. An enzyme linked immunosorbent assay (ELISA) experiment was performed in the Hip and PFC of both AD and WT mice to determine the levels of soluble Aβ in the brains of transgenic AD mice compared to their WT littermates.

2.2. Drugs and drug administration

The $GABA_A$ receptor agonist, muscimol hydrobromide, was purchased from Sigma-Aldrich (St. Louis, MO, USA) and the $GABA_A$ receptor antagonist, (+)-bicuculline, was obtained from Selleck Chemicals (Houston, TX, USA). Muscimol and bicuculline were dissolved in saline (Shandong Kangning Pharmaceutical Co., Ltd., China). Both drugs were intraperitoneally (i.p.) administered at a volume of 0.1 ml/10 g body weight at a dose of 0.1 mg/kg. The molar doses of the injected muscimol and bicuculline were 0.51 and 0.27 μmol/kg, respectively, and their molar dose ratio was 1.89. Based on our preliminary dose screening experiments (0.1, 0.5, and 1 mg/kg), muscimol improved spatial recognition memory in normal mice at a dose of 0.1 mg/kg, whereas bicuculline had no effect on memory at the same dosage. Furthermore, both drugs affected EEGs in the Hip and/or PFC at this dose. Previous studies have shown that muscimol can prevent cognitive deficits in animals at low doses of less than 1 mg/kg (Ding et al., 2015; Pilipenko et al., 2018). Thus, in the current study, we used a low dose of 0.1 mg/kg for muscimol and bicuculline. As a control, saline was administered at a volume of 0.1 ml/10 g body weight.

The experimental routine of drug administration (Fig. 1B) was randomized. WT and AD mice were used in a spontaneous EEG experiment randomly grouped for muscimol or bicuculline administration. After at least one week of drug washout, these and additional mice were used in a Y-maze EEG experiment randomly grouped for saline, muscimol, or bicuculline administration. The half-life of muscimol is relatively short (Disorbo et al., 2009; Michelot and Melendez-Howell, 2003). The drug is an active component of fly agaric and provokes poisoning when consumed, with complete recovery usually observed after 24 h without noticeable after-effects (Michelot and Melendez-Howell, 2003). The half-life of bicuculline is also relatively short (e.g., 5–6 h) (Ekimova, 2013; Johnston, 1991). The timeline of drug administration is detailed in the EEG recording protocols.

2.3. Surgery and verification of electrode locations

Surgery was performed under pentobarbital anesthesia (60 mg/kg, i.p.; dissolved in 0.9% sodium, 10 mg/ml, Merck, Darmstadt, Germany). After a midline scalp incision, five burr holes were drilled in the skull. Two twisted pairs of perfluoroalkoxy (PFA)-coated stainless steel wires (diameter 0.002", A-M systems, WA, USA) were implanted in the brain through the two holes, serving as EEG recording electrodes, to record the right Hip (AP: − 1.82 mm, ML: + 1.5 mm, DV: − 1.7 mm from skull) and right PFC (AP: + 2.95 mm, ML: + 1.5 mm, DV: − 0.75 mm from dura) (Zhang et al., 2016), respectively. A stainless-steel watch screw (M1.0 × L2.0 mm, RWD) was placed in contact with the dura through the hole, serving as the left Ctx (control) recording electrode (AP: − 2.80 mm, ML: − 2.25 mm). Through the other two holes, two stainless-steel watch screws were also placed in contact with the dura above the left olfactory bulb and central cerebellum, serving as reference and ground electrodes, respectively. All electrodes were attached to male pins that were secured in a rectangular five by one pin array and secured with dental acrylic. In addition, a general penicillin antibiotic was injected (16 000 units, i.m.; Shandong Shengwang Pharmaceutical, China) immediately after surgery. Subjects were permitted at least one week to recover from surgery.

After the EEG experiments, the locations of electrodes were confirmed histologically. Electrolytic lesions were made by applying an anode direct current (60 mA, 2–3 min) to each electrode to identify the recording locations. The animals were anesthetized with pentobarbital and intracardially perfused with saline (60–100 ml) followed by 4% paraformaldehyde (100–200 ml; Tianjin Guangfu Fine Chemical Research Institute, China). The brains were taken out and frozen sectioned at a 30-μm thickness. The electrode locations were confirmed by Nissl staining (Fig. 2). Data were excluded from any mice in which the recording locations were misplaced.

2.4. EEG recording protocols

The EEG signal acquisition system consisted of an RHD2132 amplifier, RHD2000 USB interface board, and RHD2000 Interface GUI Software (Intan Technologies, Los Angeles, CA, USA). Data were acquired at a sampling rate of 1 kHz. All electrodes on each mouse were

A

	Spontaneous EEG				Spontaneous EEG				EEG during Y-maze task				ELISA (Aβ)		
	51-52 weeks				51-52 weeks				53-55 weeks				62 weeks		
	Baseline EEG					Post-drug EEG			Behavior	EEG					
	Hip	PFC	Ctx			Hip	PFC	Ctx		Hip	PFC	Ctx		Hip	PFC
					saline				7	7	7	7			
WT	18	18	18	WT	muscimol	9	9	9	6	6	5	6	WT	9	8
					bicuculline	9	9	9	6	6	6	6			
					saline				8	7	8	7			
AD	18	20	18	AD	muscimol	9	10	9	8	7	8	7	AD	8	8
					bicuculline	9	10	9	7	6	7	7			

B

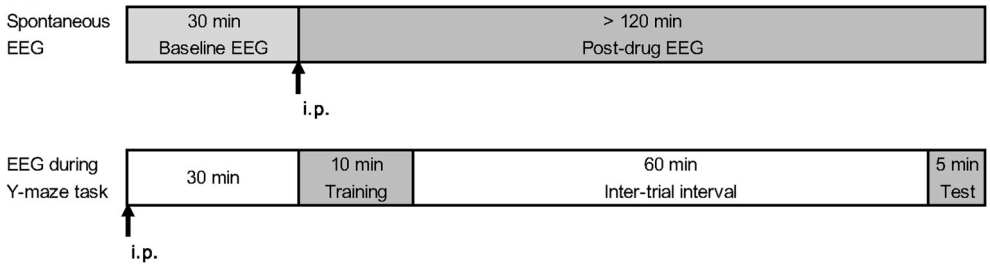


Fig. 1. Experimental design. (A) Schematic of study design and group compositions with number of animals used at different ages. (B) Timeline of drug administration in spontaneous EEG and during Y-maze EEG performance.

connected by a cable to the amplifier, then to the interface board, and finally to the computer. The cable was suspended by a helium balloon to allow the mice free movement. The recording was performed in a shielding cage (80 × 70 × 100 cm). The behaviors of mice in the cage were monitored with a ceiling-mounted CCD camera. The video signals were then displayed and saved by video-recording software.

2.4.1. EEG recording under spontaneous state

Mice were first allowed three days for habituation in the recording conditions, each for 30 min with the cable plugged in. During EEG recording, baseline activity was first acquired for 30 min, followed by an injection of muscimol, bicuculline, or saline. Post-drug EEG recordings were acquired for more than 2 h (Fig. 1B).

2.4.2. EEG recordings during Y-maze performance

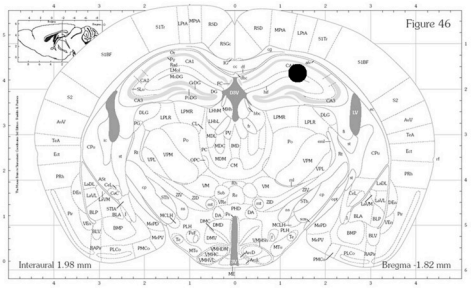
Mice were treated with muscimol, bicuculline, or saline 30 min before the Y-maze task (Fig. 1B). Video-EEG recordings were performed during the whole Y-maze task. During recording, the shielding cage was decorated with 1–2 visual spatial cues (e.g., black square, yellow circular grating, white triangle) on each of the four walls. In addition, the camera, amplifier, and exposed wood of the cage were used as spatial cues.

A Y-maze was placed in the cage. Differential visual cues were also placed on the walls of the maze. The Y-maze was made of black PVC board, and consisted of three arms with an angle of 120° between adjacent arms. Each arm was 8 × 30 × 15 cm (width × length × height). The three identical arms were randomly designated: (1) start arm, in which the mouse started to explore (always open); (2) novel arm, which was blocked during the first trial, but open during the second trial; and, (3) other arm (always open). The floor of the maze was covered with sawdust, which was mixed after each individual trial to equate differential olfactory stimuli.

The Y-maze test consisted of two trials. The first trial (training session) was of 10-min duration and allowed the mouse to explore only two arms (start and other arm) of the maze, with the third (novel) arm blocked. After an intertrial interval (ITI) of 1 h, the second trial (testing/retention session) was conducted, during which all three arms were accessible and novelty vs. familiarity was analyzed by comparing behavior in all three arms. For the second trial, the mouse was placed back in the maze in the same starting arm, with free access to all three arms for 5 min.

2.5. ELISA

The brains of mice were removed after decapitation and dissected on an ice-cold plate to isolate the Hip and PFC tissues. The tissues were initially stored in a liquid nitrogen tank and then in a refrigerator a −80 °C until further use. For protein extraction, the tissues were



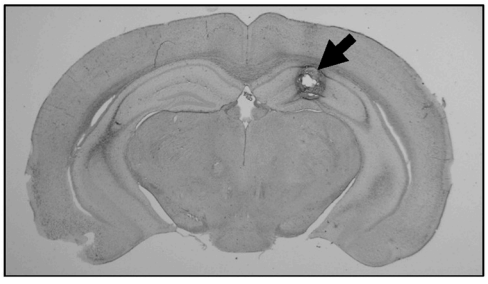


Fig. 2. Example of histological localization of lesion site. Left: schematic of anatomical location of damaged region showing electrolytic lesion on coronal section adapted from the atlas of Franklin and Paxinos. Right: representative photomicrograph of stained coronal section showing typical electrolytic lesion of hippocampus. Black dots and arrows mark lesion sites in the atlas and photomicrograph of stained coronal sections, respectively.

Table 1
Baseline EEG activity in five frequency bands from delta to gamma.

	Hip					PFC					Ctx				
RP	WT		AD		P value	WT		AD		P value	WT		AD		P value
	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
Delta	18.82	1.52	25.37	2.24	↑ < **0.05***	29.23	1.39	33.57	1.86	0.07	10.12	0.54	12.29	1.30	0.14
Theta	38.24	1.12	38.51	1.33	0.87	38.21	0.78	36.29	0.64	0.06	39.78	1.30	35.11	1.20	↓ < **0.05***
Alpha	18.06	1.01	14.42	0.66	↓ < **0.01****	13.37	0.73	11.76	0.49	0.07	20.90	1.01	19.35	1.23	0.34
Beta	13.82	0.69	13.03	1.16	0.56	13.18	1.32	12.89	1.44	0.88	14.15	0.76	19.47	2.02	↑ < **0.05***
Gamma	11.06	1.01	8.67	0.96	0.10	6.01	0.50	5.48	0.56	0.49	15.06	1.19	13.77	1.05	0.42

Up or down arrows respectively represent an increase or decrease in RP in AD mice compared to that in WT mice.
P values were obtained from comparisons between groups by Student's t-test.
Abbreviations: RP, relative power; Hip, hippocampus; PFC, prefrontal cortex; Ctx, cortex; WT, wild type; AD, APP and PS1 double transgenic mice.

homogenized in RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCL, pH 8.0) containing protease and phosphatase inhibitors (Thermo Scientific, Rockford, IL, USA). Homogenates were then centrifuged at 3 000 rpm for 20 min at 4 °C. The supernatants were collected and stored at −80 °C for ELISA. The levels of soluble Aβ in the Hip and PFC of mice were determined by a total human Aβ ELISA kit (EH025-48; ExCell Biology, Shanghai, China) in accordance with the manufacturer's protocols. Absorbance was measured at 450 nm using a 96-well plate reader. The concentrations of total Aβ were calculated from standard curves, and data were expressed as pg/ml.

2.6. Data analysis

As described earlier (Fu et al., 2008, 2011), EEG signals were examined by off-line analysis and spectral analysis was performed by MATLAB. EEGs were recorded by the Intan system as rhd data files: spontaneous EEGs were saved every 10 min; Y-maze EEGs were saved with every entry into each arm. Each rhd data file was first separated into segments, with each segment comprised of 1 024 sample points. After computer-assisted rejection of segments with artifacts, each segment was filtered for the following EEG frequency bands (with no 50 Hz notch filter): (1) delta: 2–4 Hz, (2) theta: 4–8 Hz, (3) alpha: 8–12 Hz, (4) beta: 12–20 Hz, and (5) gamma: 20–100 Hz. For each frequency band, the absolute power of each segment was calculated as: $P = \sum X^2/1\,024$. The relative power (RP) was calculated as the percentage of power relative to the total power of all frequency bands.

Spontaneous EEG analysis was performed as follows. Baseline EEG activity was calculated as the mean value of RP during 30 min of recording. To analyze EEG activity in post-drug recordings, normalized RP (NRP) was used and calculated as: NRP = (RP1 − RP0)/RP0 × 100%, (RP0: RP for baseline recording; RP1: RP for post-drug recording). To obtain EEG changes with time, the NRP data for every 10 min of recording were analyzed first. Two indices were then calculated: (1) mean value of NRP of the whole recording time; and, (2) peak value of NRP during the entire recording time, which was calculated as the maximal NRP absolute value. For example, for the set of values (post-drug recording: 10–130 min) 5.47, −4.05, −10.08, −10.46, −13.50, −24.47, −23.40, −19.38, −19.97, −15.60, −14.67, −24.08, and −16.52, the maximal NRP absolute value was 24.47, so the peak value was −24.47.

Y-maze task-related EEG analysis was performed as follows. The mean values of RP were separately calculated for the 10-min training sessions and 5-min testing sessions. The mean RP of the testing sessions consisted of EEG data recorded in the novel and familiar arms of the Y-maze. EEG data in the start arm were excluded to avoid the stimuli of placing the animal in the maze. Behavioral data in the Y-maze were expressed as: (1) Duration of arm visits, i.e., time spent in each arm; (2) Number of arm visits, i.e., number of entries in each arm; and (3) Total number of arm entries in the Y-maze. Data from (1) and (2) were spatial recognition memory indices, whereas (3) was a locomotor activity index, all of which were determined during the 5-min retention test.

Comparisons of EEG baseline activity between the two groups were analyzed using Student's *t*-test. Spontaneous EEG activity with time was analyzed by repeated-measures ANOVA (ANOVA-R). Comparison of baseline activity for each group was performed using ANOVA-R, whereas group differences were determined by multivariate ANOVA-1. Y-maze task-related EEG activity was analyzed by two-way ANOVA (ANOVA-2). Further analysis of group differences was determined by Student's *t*-test and treatment differences were determined by ANOVA-1 where appropriate. For behavioral data, differences between groups were assessed with ANOVA-R and ANOVA-1 where appropriate, and comparison with chance level (33.3%) was analyzed by one-sample *t*-test. In addition, comparisons of Aβ levels between the two groups were analyzed using Student's *t*-test. Data were expressed as means ± SEM. *P*-values of <0.05, <0.01, and <0.001 were considered significant, highly significant, and very highly significant, respectively.

3. Results

3.1. Spontaneous EEG recordings

3.1.1. AD mice exhibited abnormal baseline EEG activity

Each animal was recorded for 30 min before drug administration to obtain baseline EEG activity. The RPs of all five frequency bands from all three recording regions were analyzed and then compared between AD and WT groups (Table 1). The RP of the Hip EEG showed a significant increase in the delta band and significant decrease in the alpha band in AD mice compared to WT mice ($t(34) = -2.43$ and 3.02; $P < 0.05$ and <0.01 for delta and alpha, respectively). For the PFC region, there were no significant differences in RP among all frequency EEG bands for AD mice with respect to WT mice ($P \geq 0.05$ for all). In addition, the Ctx region showed significantly lower and higher RP in the theta and beta bands, respectively, in AD mice compared with WT mice ($t(34) = 2.64$ and -2.46 for theta and beta, respectively; $P < 0.05$ for both).

3.1.2. $GABA_A$ activation and inhibition altered spontaneous EEG activity in AD mice

Following drug administration, each animal was recorded for spontaneous EEG activity for more than 2 h. The NRP from baseline activity for all EEG frequency bands was analyzed, and the mean and peak values within the whole recording time were calculated and compared between AD and WT mice (Table 2). The peak value reflects the maximal changes from baseline activity during the recording period.

Muscimol treatment induced EEG activity differences between groups in several frequency bands (delta and alpha). Significant differences were observed in the PFC but not in the Hip or Ctx regions. In the PFC, mean delta EEG activity was significantly lower ($t(17) = 2.21$,

Table 2
Post-drug spontaneous EEG activity in five frequency bands from delta to gamma.

NRP		Hip					PFC					Ctx				
		WT		AD		P value	WT		AD		P value	WT		AD		P value
		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
Mean values																
Muscimol	**Delta**	0.19	5.52	−4.88	4.71	0.50	7.06	4.86	−5.29	2.99	↓<**0.05***	30.16	8.56	14.83	8.40	0.22
	Theta	2.83	2.31	4.40	2.80	0.67	0.25	2.08	6.68	3.25	0.12	4.45	2.70	2.70	4.18	0.73
	Alpha	5.78	7.77	9.17	5.84	0.73	0.69	7.50	12.46	6.87	0.26	1.11	5.42	9.18	8.81	0.45
	Beta	9.60	5.58	2.72	4.58	0.35	−4.26	5.68	−4.44	5.17	0.98	1.42	3.34	2.00	4.14	0.92
	Gamma	−11.87	4.87	−11.10	6.18	0.92	−14.02	5.73	−11.09	8.79	0.79	−24.33	6.27	−4.64	11.28	0.15
Bicuculline	**Delta**	10.49	8.49	−11.98	6.97	0.06	28.65	14.42	−5.78	6.41	↓<**0.05***	24.76	10.79	16.28	9.41	0.56
	Theta	1.25	2.67	0.66	2.76	0.88	2.20	2.87	1.89	2.27	0.93	0.69	2.10	4.55	2.60	0.26
	Alpha	5.27	5.70	20.61	6.36	0.09	−6.66	8.82	20.26	7.75	↑<**0.05***	6.92	3.90	13.16	8.30	0.51
	Beta	2.42	5.21	23.85	10.08	0.08	−16.81	8.85	17.44	14.72	0.07	4.42	4.80	−4.00	4.97	0.24
	Gamma	−14.04	5.91	15.19	10.15	↑<**0.05***	−23.79	9.71	11.15	13.16	0.05	−21.11	7.20	−17.68	4.49	0.69
Peak values																
Muscimol	**Delta**	2.07	14.48	−4.71	13.86	0.74	18.29	17.37	−10.35	9.14	0.15	72.15	16.14	51.77	19.33	0.43
	Theta	5.59	6.59	7.53	6.58	0.84	5.34	8.44	17.76	4.62	0.20	10.11	6.07	5.64	8.27	0.67
	Alpha	10.93	14.79	23.78	16.75	0.57	−7.74	15.87	36.67	13.74	↑<**0.05***	7.80	12.13	21.35	15.68	0.50
	Beta	22.18	10.59	7.28	12.07	0.37	14.78	20.63	−11.36	13.99	0.31	8.07	9.17	4.75	10.61	0.82
	Gamma	−24.96	12.42	−2.82	14.02	0.26	−41.94	14.65	6.35	24.77	0.12	−52.54	8.98	−20.10	21.76	0.19
Bicuculline	**Delta**	35.67	19.12	−21.73	15.02	↓<**0.05***	57.69	28.17	−9.64	14.30	↓<**0.05***	69.88	20.81	49.22	19.50	0.48
	Theta	1.74	6.57	4.86	6.96	0.75	10.54	9.32	1.62	7.29	0.46	1.73	6.96	10.99	6.21	0.34
	Alpha	16.58	12.38	51.38	16.01	0.11	−13.39	14.84	55.23	15.56	↑<**0.01****	20.34	9.59	28.92	16.41	0.66
	Beta	9.85	11.38	69.54	20.09	↑<**0.05***	−26.38	14.73	51.61	26.89	↑<**0.05***	6.23	10.96	−1.21	10.67	0.63
	Gamma	−33.01	12.80	59.76	26.27	↑<**0.01****	−40.10	17.57	61.62	27.91	↑<**0.01****	−34.70	15.90	−50.37	4.52	0.37

Up or down arrows respectively represent an increase or decrease in NRP in AD mice compared to that in WT mice.
P values were obtained from comparisons between groups by Student's t-test.
Abbreviations: NRP, normalized relative power, Hip, hippocampus; PFC, prefrontal cortex; Ctx, cortex; WT, wild type; AD, APP and PS1 double transgenic mice.

$P < 0.05$) and peak alpha EEG activity was significantly higher ($t(17) = -2.13$, $P < 0.05$) in AD than in WT mice.

In contrast, bicuculline treatment led to EEG activity differences between groups in a wider range of bands (from delta to gamma, excluding theta) in both the PFC and Hip regions but not in the Ctx region (Table 2). In the PFC and Hip regions, the mean and/or peak RP values were significantly lower in the delta band but significantly higher in the alpha-gamma bands for AD mice than for WT mice (Hip mean: $t(16) = -2.49$, $P < 0.05$ for gamma; PFC mean: $t(17) = 2.26$ and -2.30 for delta and alpha, respectively, $P < 0.05$ for both; Hip peak: $t(16) = 2.36$, -2.59, and -3.17, $P < 0.05$, <0.05, and <0.01 for delta, beta, and gamma, respectively; PFC peak: $t(17) = 2.20$, -3.17, -2.46, and -3.00, $P < 0.05$, <0.01, <0.05, and <0.01 for delta, alpha, beta, and gamma, respectively). For these four frequency bands, Fig. 3 shows changes in the Hip and PFC EEG activities with time. Time had a significant effect on EEG activities of Hip delta and alpha-beta bands and PFC delta and alpha bands (Hip: $F(13, 208) = 2.96$, 4.64, and 4.34, $P < 0.05$, 0.01, and 0.01 for delta, alpha, and beta, respectively; PFC: $F(13, 221) = 2.69$ and 4.14, $P < 0.05$ and 0.01 for delta and alpha, respectively). Furthermore, a significant Group effect was found on Hip gamma and PFC delta, alpha, and gamma bands (Hip gamma: $F(1, 16) = 6.08$, $P < 0.05$; PFC: $F(1, 17) = 5.30$, 5.43, and 4.53, $P < 0.05$, <0.001, and <0.05 for delta, alpha, and gamma, respectively). Further analysis was performed on each group and time point in each band, with significance marked in the figures. Briefly, the changes in EEG activities shown in the figure were in accordance with the mean and peak values in Table 2. In total, bicuculline treatment decreased EEG activities in the delta band and increased EEG activities from the alpha to gamma bands in AD mice compared to those in WT mice.

3.2. Y-maze performance EEG recordings

3.2.1. $GABA_A$ activation and inhibition improved memory retention in AD mice

Behavioral data were first analyzed. Combined ANOVA-R displayed significant differences in the percentages of time spent and number of visits in the three arms of the Y maze ($F(2,72) = 9.53$ and 9.34 for Time and Number, respectively, $P < 0.001$ for both). Further analysis was then performed for each group and treatment. WT mice possessed good memory retention, but AD mice did not, as found after saline treatment (Fig. 4A and B, left columns). The percentage of number of visits in the novel arm was significantly lower for AD mice than for WT mice ($P < 0.05$). For the WT group, there was a significant Arm effect on the percentage of number of visits ($F(2, 12) = 11.86$, $P < 0.01$), with the percentage in the novel arm also significantly higher than chance level (33.3%; $P < 0.01$).

Muscimol treatment improved memory performance in AD mice (Fig. 4A and B, middle columns). For this group, muscimol treatment significantly increased the percentage of time spent in the novel arm compared with that of the saline treatment (Treatment effect, $F(2,20) = 6.64$, $P < 0.01$; muscimol vs. saline, $P < 0.05$). In addition, both AD and WT mice had scores significantly higher than chance levels for the percentage of time spent and/or the percentage of number of visits in the novel arm ($P < 0.05$ for all).

Bicuculline treatment also improved memory retention in AD mice but showed impairment in WT mice (Fig. 4A and B, right columns). The Arm effect was significant for the percentages of time spent and number of visits in AD mice ($F(2, 12) = 12.00$ and 4.53, $P < 0.001$ and 0.05 for Time and Number, respectively). AD mice also had scores significantly higher than chance for the percentages of time spent and number of visits ($P < 0.05$ for both). In addition, bicuculline treatment significantly increased the percentage of time spent in the novel arm in AD mice compared with that of saline treatment ($P < 0.01$). It is worth

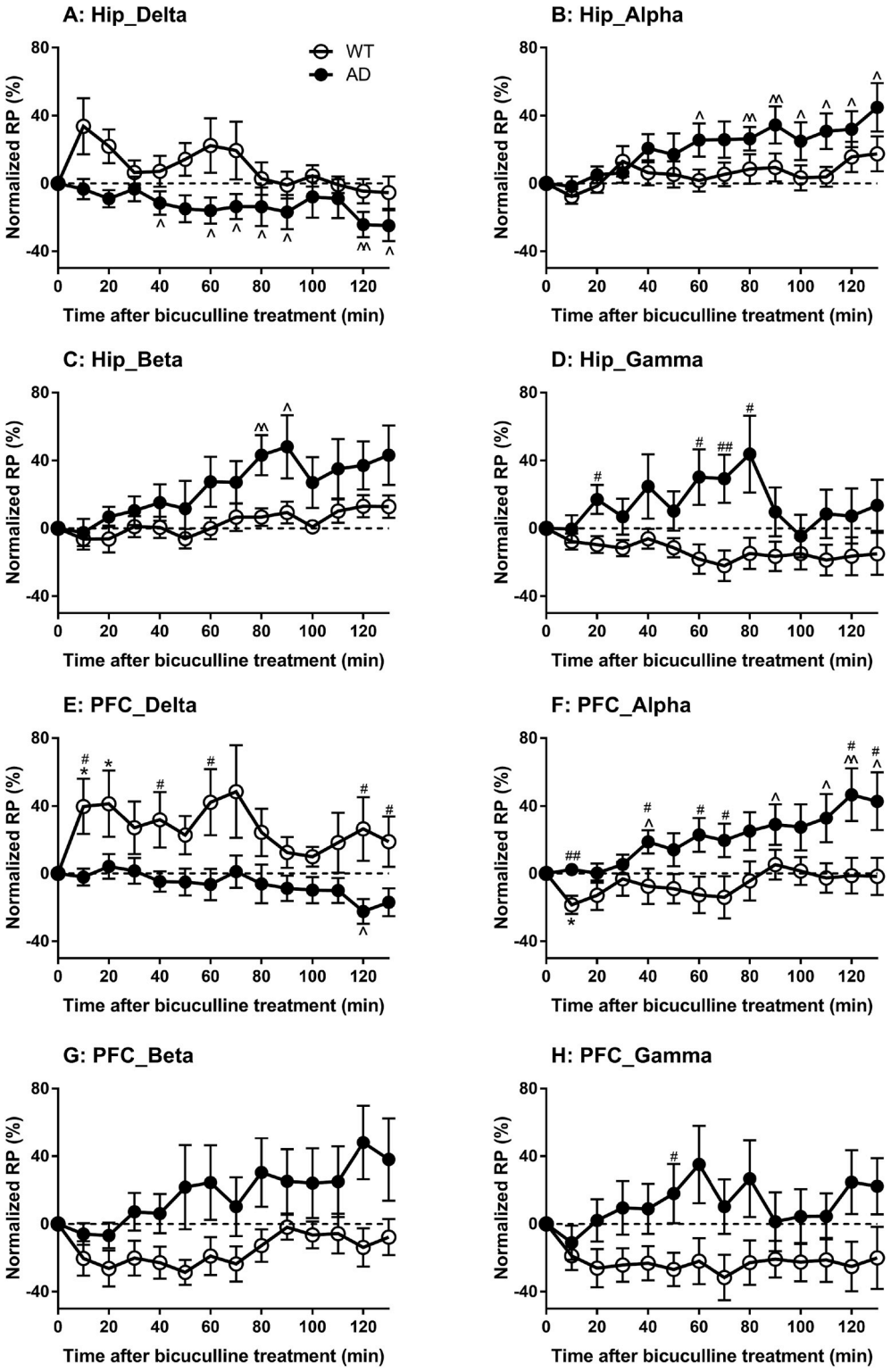


Fig. 3. Effects of bicuculline treatment on normalized relative power in all EEG frequency bands, except theta, in AD and WT mice. Hippocampus (A–D) and prefrontal cortex (E–H) EEG activities were recorded for more than 2 h. Dashed line indicates baseline EEG activity. $^{\wedge}P < 0.05$ and $^{\wedge\wedge}P < 0.01$ compared with baseline activity for AD group; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with baseline activity for WT group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ comparison between AD and WT groups. Abbreviations: Hip, hippocampus; PFC, prefrontal cortex; RP, relative power.

noting that none of these analyses showed significance in WT mice ($P > 0.05$ for all).

For locomotor activity in the Y-maze, there were no significant differences between the genotype groups ($F(1, 38) = 0.08$, $P = 0.79$) or among drug treatments ($F(2, 38) = 0.15$, $P = 0.86$) (Fig. 5).

3.2.2. $GABA_A$ activation and inhibition altered Y-maze EEG activity in AD mice

EEG activity was recorded while mice performed the Y-maze task, for both the training and testing sessions. The RP for each frequency band was analyzed and compared between groups and among

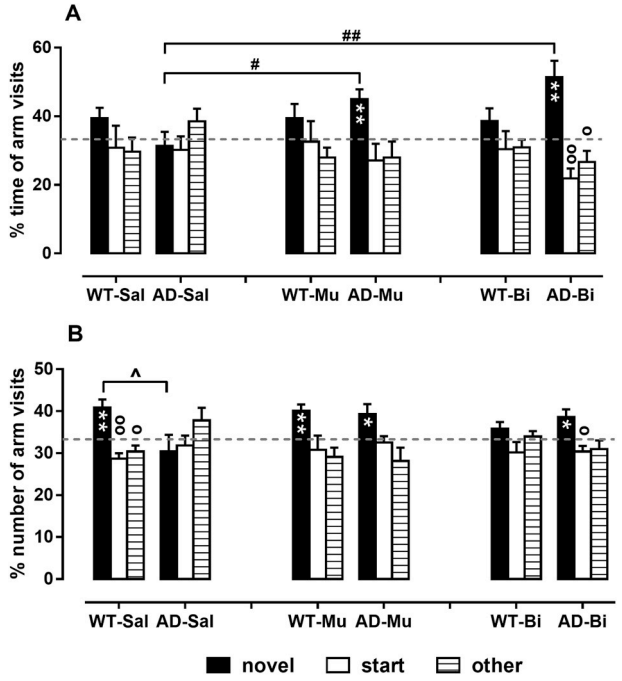


Fig. 4. Effects of muscimol and bicuculline treatment on spatial recognition memory in Y-maze task in AD and WT mice. Percentages of time spent (A) and visits (B) to three arms during 5-min retrieval trial. Dashed line indicates chance level (33.3%). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with chance level; $^{o}P < 0.05$ and $^{oo}P < 0.01$ compared with novel arm in each group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ comparison between muscimol, bicuculline, and saline treatment in AD and WT mice; $\hat{}P < 0.05$ comparison between AD and WT mice. Abbreviations: Sal, saline; Mu, muscimol; Bi, bicuculline.

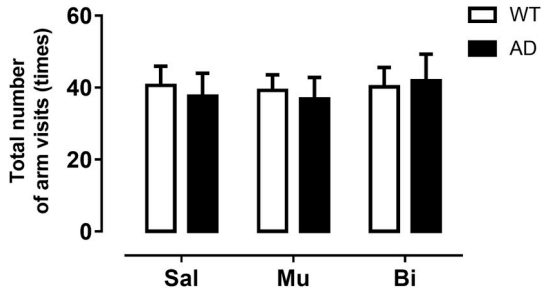


Fig. 5. Effects of muscimol and bicuculline treatment on locomotor activities in Y-maze task in AD and WT mice. Abbreviations: Sal, saline; Mu, muscimol; Bi, bicuculline.

treatments.

Muscimol treatment decreased PFC EEG activity in the delta band but increased PFC EEG activity in the theta band in AD mice during the Y-maze task (Fig. 6). EEG activity was significantly lower in the delta band and significantly higher in the theta band after muscimol treatment than after saline treatment in both task sessions in AD mice (Delta in training: $F(2,20) = 4.70$, $P < 0.05$ and Mu vs. Sal, $P < 0.05$; Theta in training: $F(2,20) = 7.76$, $P < 0.01$ and Mu vs. Sal, $P < 0.01$; Delta in testing: $F(2,20) = 3.60$, $P < 0.05$ and Mu vs. Sal, $P < 0.05$; Theta in testing: $F(2,20) = 4.40$, $P < 0.05$ and Mu vs. Sal, $P < 0.05$), which was not observed in WT mice ($P > 0.05$ for all). For the Hip region, the EEG activity for all frequency bands showed no significant differences between groups or among treatments for either task session ($P > 0.05$ for all).

Bicuculline treatment led to EEG differences in a wider range of bands (from delta to gamma) between groups and among treatments during Y-maze performance, especially in the PFC region (Fig. 6). Compared with WT mice, AD mice showed significantly decreased RP in the delta band in both task sessions (Training: $t(11) = 3.95$, $P < 0.01$; Testing: $t(11) = 4.99$, $P < 0.001$). In addition, AD mice showed significantly increased RP in the high frequency bands, i.e., from theta to beta bands during the training session ($t(11) = -2.82$, -2.55, and -3.98, $P < 0.05$, <0.05, and <0.01 for theta to beta, respectively), and from alpha to gamma bands during the testing session ($t(11) = -4.36$, -4.81, and -2.85, $P < 0.01$, <0.01, and <0.05 for alpha to gamma, respectively). Interestingly, when analyzing differences between treatments, AD mice demonstrated different EEG changes to those of WT mice. For AD mice, the RP was lower in the delta band and higher in the theta band after bicuculline treatment than after saline treatment during the training session (Delta: $F(2,20) = 4.70$, $P < 0.05$ and Bi vs. Sal, $P < 0.01$; Theta: $F(2,20) = 7.76$, $P < 0.01$ and Bi vs. Sal, $P < 0.01$). For WT mice, higher delta RP and lower theta RP were observed after bicuculline treatment than after saline treatment during the testing session (Delta: $F(2,15) = 11.62$, $P < 0.01$ and Bi vs. Sal, $P < 0.01$; Theta: $F(2,15) = 5.82$, $P < 0.05$ and Bi vs. Sal, $P < 0.01$). Conversely, Hip EEG activity exhibited similar differences in the delta band as observed in the PFC region between the two groups (Training: $t(11) = 2.79$, $P < 0.05$; Testing: $t(10) = 3.35$, $P < 0.01$).

The above analyses after muscimol and bicuculline treatment were also applied to the Ctx region in AD and WT mice (data not shown). The only significant difference in EEG activity was found in the beta band after muscimol treatment, i.e., AD mice exhibited significantly higher RP than WT mice during the training session ($t(11) = -2.97$, $P < 0.05$).

3.3. Summary of EEG activity data in AD mice

Table 3 summarizes the spontaneous and task-related EEG alterations in five frequency bands in the Hip and PFC of AD mice. The AD mice showed an increase in the delta band and decrease in the alpha band in Hip EEG baseline activity, as well as a decrease in the theta band in PFC EEG baseline activity. $GABA_A$ activation by muscimol mainly affected PFC EEG activity in AD mice, with a decrease in the delta band and increase in the theta-alpha bands. In contrast, $GABA_A$ inhibition by bicuculline impacted EEG activity in both the Hip and PFC, reflected by a decrease in the delta band and increase in the beta-gamma bands. PFC theta and alpha EEG activities also increased after this inhibition.

Both types of $GABA_A$ intervention induced similar spontaneous and task-related EEG activity changes in the PFC, i.e., delta activity deceased and alpha activity increased under the spontaneous state, and delta activity deceased and theta activity increased during Y-maze performance.

3.4. Total Aβ was higher in AD brains

In AD mice, total Aβ concentrations were 4.44 ± 0.53 pg/ml for the Hip and 4.99 ± 0.20 pg/ml for the PFC, which were significantly higher than the 0.11 ± 0.01 pg/ml for the Hip and 0.07 ± 0.00 pg/ml for the PFC in WT mice (Hip: $t(15) = -8.14$, $P < 0.001$; PFC: $t(14) = -24.61$, $P < 0.001$) (Fig. 7).

4. Discussion

Our results provide the first evidence that slight activation and inhibition of $GABA_A$ receptors restore EEG activities in aged APP and PS1 double transgenic mice and improve their spatial recognition memory. AD mice exhibited slower brain activity, with $GABA_A$ receptor intervention reversing this alteration, as judged by changes in EEG activity in the delta-theta frequency bands in the PFC and/or Hip. This activation and inhibition also led to different EEG activity changes in the PFC and Hip, indicating specific differences in the related neural mechanisms.

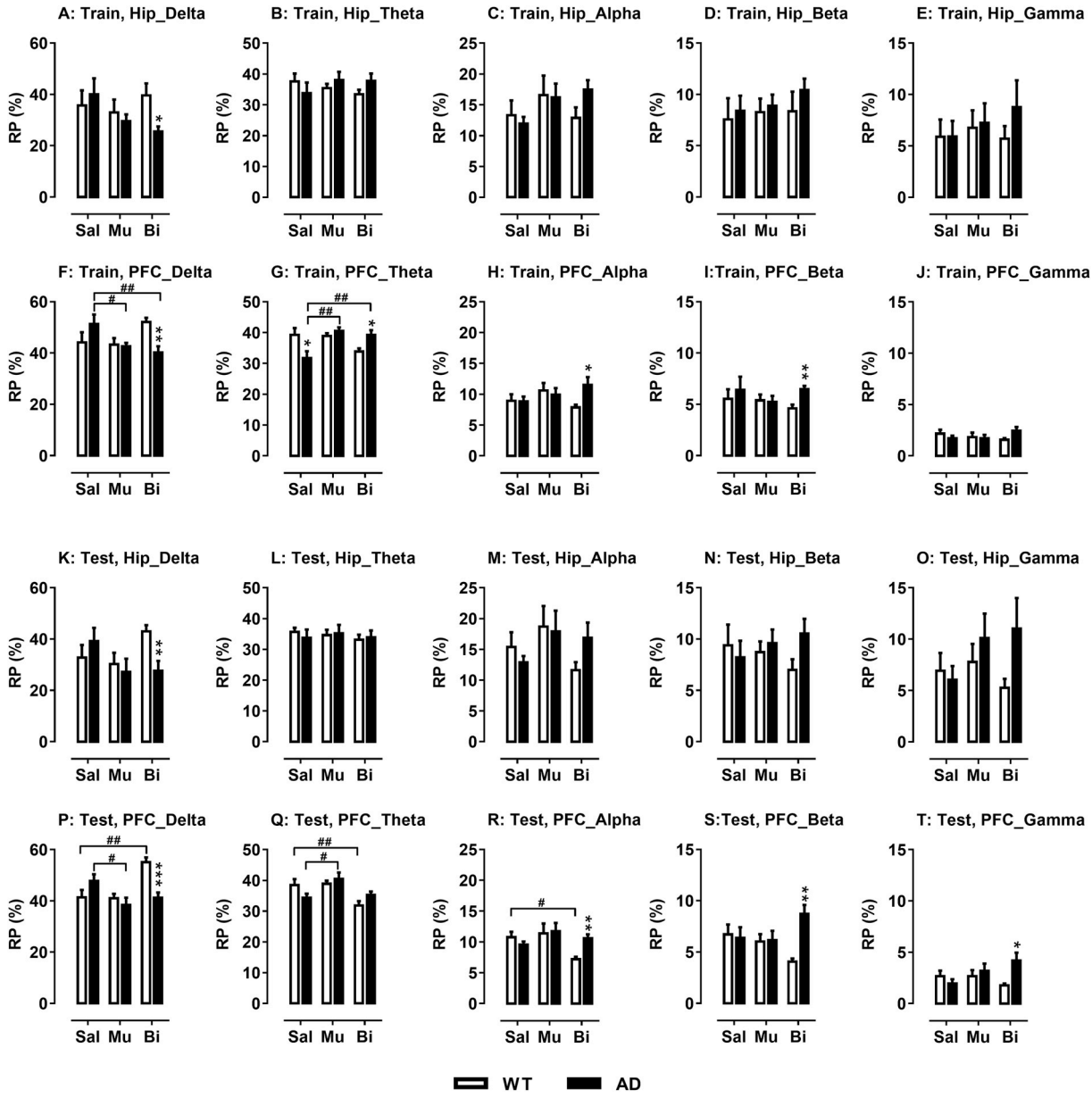


Fig. 6. Effects of muscimol and bicuculline treatment on relative power in five EEG frequency bands in AD and WT mice during Y-maze task performance. Hippocampus (A-E and K–O) and prefrontal cortex (F-J and P-T) EEG activities recorded during training (A–J) and testing phases (K–T) of task. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ comparison between AD and WT groups during each drug treatment. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ comparison between muscimol, bicuculline, and saline treatment in AD or WT mice. Abbreviations: Train, training phase; Test, testing phase; Hip, hippocampus; PFC, prefrontal cortex; Sal, saline; Mu, muscimol; Bi, bicuculline; RP, relative power.

In the current study, basal EEG activity in AD mice showed significantly increased delta activity and decreased alpha activity in the Hip. Previous studies on transgenic AD mice and rats with Aβ fragment injections have also demonstrated an increase in global or Hip EEG delta activity (Del Percio et al., 2018; Dringenberg, 2000; Hidisoglu et al., 2018; Jyoti et al., 2010; Platt et al., 2011; Schneider et al., 2014) and decrease in EEG alpha activity (Ganguly and Guha, 2008; Koss et al., 2016). Our results are in accordance with these previous studies exhibiting slower brain activity in AD.

We also found a decrease in PFC theta activity in AD mice under spontaneous state and during Y-maze performance. This agrees with previous research, in which AD mice showed reduced frontoparietal theta activity during cage exploration (Del Percio et al., 2018). In addition, EEG activity in the theta band also showed alterations in AD rodents during the resting state (e.g., hippocampal or cortical EEG) (Gurevicius et al., 2013; Jyoti et al., 2010; Papazoglou et al., 2016; Schneider et al., 2014; Wang et al., 2002). Conversely, several studies have found no changes of this frequency band (e.g., hippocampal, prefrontal, cortical, or parietal EEG) (Liu et al., 2014; Papazoglou et al., 2017; Zhang et al., 2016). These differences in outcome may depend on experimental variables, such as animal models and recording sites, as well as the wakeful states of animals.

In this study, AD mice exhibited impaired spatial recognition memory in the Y-maze task, as reflected by the decreased percentage of visits to the novel arm. The Y-maze paradigm is based on a rodent's natural tendency to explore novelty and is a simple two-trial recognition test for measuring spatial recognition memory (Fu et al., 2017). Commonly, the behavioral paradigms for assessing this memory are by Morris water maze and Barnes maze. These paradigms, however, involve punishments (e.g., falling into water, strong light) that may have

Table 3
Summary of EEG activities in AD mice.

		Spontaneous EEG			Task-related EEG					
					Training session			Testing session		
		Baseline	Muscimol	Bicuculline	Saline	Muscimol	Bicuculline	Saline	Muscimol	Bicuculline
Hip	**Delta**	++		--			--			--
	Theta									
	Alpha	--								
	Beta			++						
	Gamma			++						
PFC	**Delta**		--	--		--	--		--	--
	Theta				--	++	++		++	
	Alpha		++	++			++			++
	Beta			++			++			++
	Gamma			++						++

Data were compared with WT mice or the same genotype mice but with saline injection.
Black double pluses or minuses represent significant changes ($P < 0.05$).

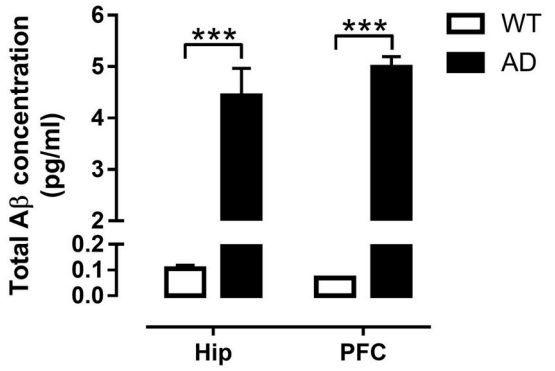


Fig. 7. Total Aβ concentrations in Hip and PFC of AD and WT mice. ***$P < 0.001$ comparison between AD and WT groups. Abbreviations: Hip, hippocampus; PFC, prefrontal cortex.

nonspecific effects. Thus, our study provides evidence that spatial recognition memory unrelated to reward or punishment was still impaired in AD mice.

Interestingly, the AD mice treated with muscimol and bicuculline showed improved spatial recognition memory in the Y-maze task. These findings are in accordance with several previous studies showing that low doses of both drugs improve memory function in normal and AD rodents. For example, at very low doses of 0.01–0.05 mg/kg, muscimol can enhance spatial memory in AD rats (Pilipenko et al., 2018). In addition, bicuculline at doses below 0.1–0.3 mg/kg can enhance memory retention of aversively motivated tasks in normal mice (Brioni and McGaugh, 1988). Together, these data indicate that even slight $GABA_A$ receptor intervention has implications for memory restoration in AD rodent models.

The improvements in Y-maze spatial cognition in our AD mice were accompanied by decreased EEG delta activity and increased EEG theta activity in the PFC. The decrease in delta band activity in AD mice was also observed under the spontaneous recording state. Thus, $GABA_A$ receptor intervention caused a shift in neural network activity towards higher oscillatory rhythm, leading to faster EEG brain activity. As discussed above, previous research and our data indicated slower EEG activity in the AD brain. Thus, it could be concluded that slight $GABA_A$ activation and inhibition restored EEG activity in AD mice. Altered EEG activity can lead to cognitive abnormalities in AD models (Palop and Mucke, 2016), and EEG recovery may explain improved memory retention.

An apparent paradox is that muscimol is a $GABA_A$ agonist and bicuculline is a $GABA_A$ antagonist, but both had similar effects on spatial memory in our AD mice. A possible explanation is that while muscimol is widely used as a selective $GABA_A$ agonist, it actually exhibits more potent action as a partial agonist at $GABA_C$ receptors (Johnston, 2014). Previous research has indicated that $GABA_A$ and $GABA_C$ receptors have opposing actions on short-term memory formation in chicks (Gibbs and Johnston, 2005). Muscimol may activate both $GABA_A$ and $GABA_C$ receptors together, whereas bicuculline blocks only $GABA_A$ receptors, thus confounding the opposing involvement of these receptors on some aspects of memory. It would be interesting, in the future, to determine whether the effects of muscimol can be completely blocked by $GABA_A$ antagonists, and whether these effects are insensitive to $GABA_C$ antagonists. Such research could help clarify whether muscimol affects memory based solely on the involvement of $GABA_A$ receptors in AD mice.

In addition, accumulating evidence suggests that $GABA_A$ agonists and antagonists can benefit both normal and AD brains but with different neural mechanisms. $GABA_A$ agonists have neuroprotective effects (Nava-Mesa et al., 2014). For example, muscimol can inhibit neurotoxicity, reduce cell death, and protect the Hip from synaptic damage and apoptosis (Ding et al., 2015). Muscimol also inhibits Aβ-induced apoptotic death in cultured rat cortical cells (Lee et al., 2005) and demonstrates anti-inflammatory effects, normalization of acetylcholine esterase and GABA expression, and memory enhancement in AD rats *in vivo* (Pilipenko et al., 2018). The benefits of $GABA_A$ antagonists in AD relate to the restoration of synaptic plasticity, compensation for the dampening of hyperexcitation, and cooperation with the cholinergic system (Li et al., 2016; Palop et al., 2007). For example, application of $GABA_A$ antagonist picrotoxin can prevent long-term potentiation (LTP) deficits in animal models of AD (Kleschevnikov et al., 2004), whereas bicuculline treatment can result in LTP-like facilitation in the dentate gyrus of C57BL/6 and AD transgenic mice (Matsuyama et al., 2008). In addition, as a competitive $GABA_A$ antagonist and acetylcholinesterase inhibitor, bis(7)-tacrine can enhance memory and may be a potential palliative therapeutic agent for AD.

On the other hand, although muscimol and bicuculline had similar effects on EEG delta-alpha activities in the PFC of AD mice, both exhibited specific EEG activity differences in this region as well as in the Hip. In the PFC, bicuculline increased EEG activities in the higher frequency bands in AD mice (i.e., beta-gamma under spontaneous state and alpha-gamma during Y-maze task), which were not observed after muscimol treatment. In the Hip, bicuculline treatment caused significant changes in EEG activity across a wider range of bands in AD mice (except theta-alpha), which were not found with muscimol treatment.

The specific regional EEG activity differences between muscimol and bicuculline treatments could be attributed to their distinct

pharmacological profiles. Muscimol exhibits poor blood-brain barrier penetrability due to its relatively low lipid solubility (Maggi and Enna, 1979), whereas bicuculline exhibits high lipid solubility and penetrability (Engstrom and Woodbury, 1988). In addition, muscimol is a potent agonist at $GABA_A$ receptors and a potent partial agonist at $GABA_C$ receptors, and the action of this chemical agent on different subunit combinations of most $GABA_A$ receptors is relatively uniform (Johnston, 2014). In contrast, bicuculline acts as an antagonist at $GABA_A$ receptors and is inactive at $GABA_C$ receptors, and its potency is largely independent of receptor subunit composition (Johnston, 2013).

Currently, accumulating evidence suggests that AD pathology involves enhancement in tonic inhibition, such as up-regulation of $GABA_A$ receptors containing the α5 subunit (Li et al., 2016; Wu et al., 2014). Reducing tonic inhibition by blockade of this receptor subtype can rescue LTP impairment and alleviate memory deficits in AD mice (Wu et al., 2014). Thus, it is possible that bicuculline, as a $GABA_A$ antagonist, improved memory in our AD mice by reducing tonic inhibition among neurons. However, the competitive antagonism of bicuculline is suggested to target mainly α1 $GABA_A$ receptors (Huang et al., 2003). Thus, further evidence is required to confirm if other tonic inhibitory mechanisms other than the α5 receptor subtype are involved in the pharmacology of bicuculline in the AD brain.

Previous studies have observed an E/I imbalance in AD rodents (Lei et al., 2016; Leon-Espinosa et al., 2012; Palop et al., 2007). For example, very low level soluble Aβ can disrupt Glu/GABA balance, increase neuronal excitability, and impair synaptic plasticity (Lei et al., 2016). In addition, Aβ plaques decrease GABAergic innervation and result in imbalance between excitatory and inhibitory inputs (Leon-Espinosa et al., 2012). Thus, maintaining an intrinsic E/I balance is considered a potential strategy for AD treatment (Graf and Kater, 1998). Several clinical drugs, such as memantine, shift the E/I balance from inhibition in brain regions including the PFC (Povysheva and Johnson, 2016). Our study provides evidence that modulation of E/I balance contributes to recovery of brain function in AD mice.

The age of the AD mice in the current study was around 12 months. These mice developed Aβ deposits in the brain at 6–7 months of age (Wang et al., 2015), and our data showed evident Aβ pathology at the brain level. Thus, our mice were at a very late stage of AD. Previous studies have suggested that maintaining a balance between E/I neurotransmitter systems constitutes a promising therapeutic approach for treatment at the early pre-dementia stage of AD (Nava-Mesa et al., 2014; Palop and Mucke, 2010, 2016). Thus, it would be of interest to test the role of $GABA_A$ in younger AD mice before and at the onset of the disease, such as at 3–4 and 6–7 months old, respectively.

There is a concern that the observed differences in EEG activity between AD and WT mice may be due to different effects of muscimol and bicuculline on the sleep-wake pattern. However, during the Y-maze EEG experiment, the activities of mice were monitored and evaluated by indices such as the total number of arm visits. Our data showed no significant differences in this index between AD and WT groups as well as among different drug treatments. Thus, the EEG differences between groups could not result from different behaviors such as sleep-wake patterns. As similar EEG differences (e.g., delta band) were observed after drug treatment between spontaneous and Y-maze EEGs, we supposed that the spontaneous EEG results were not merely due to the different effects of drugs on the sleep-wake pattern. Thus, it would be interesting to investigate how $GABA_A$ drugs affect sleep-wake EEG activity in AD animals in future studies.

In summary, we found that $GABA_A$ receptor intervention by low-dose muscimol and bicuculline application restored EEG activity and improved spatial recognition memory in aged APP and PS1 double transgenic mice. Our study highlights the potential clinical use of $GABA_A$ agonists and antagonists to improve cognitive disorders and restore neural network activity in AD.

Conflicts of interest

The authors declare there are no conflicts of interest.

Acknowledgments

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References

Abbott, A., Dolgin, E., 2016. Failed Alzheimer's trial does not kill leading theory of disease. Nature 540, 15–16.
Anand, R., Gill, K.D., Mahdi, A.A., 2014. Therapeutics of Alzheimer's disease: past, present and future. Neuropharmacology 76 Pt A, 27–50.
Brioni, J.D., McGaugh, J.L., 1988. Post-training administration of GABAergic antagonists enhances retention of aversively motivated tasks. Psychopharmacology (Berlin) 96, 505–510.
Castellano, C., Introini-Collison, I.B., McGaugh, J.L., 1993. Interaction of beta-endorphin and GABAergic drugs in the regulation of memory storage. Behav. Neural. Biol. 60, 123–128.
Del Percio, C., Drinkenburg, W., Lopez, S., Limatola, C., Bastlund, J.F., Christensen, D.Z., Pedersen, J.T., Forloni, G., Frasca, A., Noe, F.M., Bentivoglio, M., Fabene, P.F., Bertini, G., Colavito, V., Dix, S., Ferri, R., Bordet, R., Richardson, J.C., Babiloni, C., 2018. Ongoing electroencephalographic activity associated with cortical arousal in transgenic PDAPP mice (hAPP V717F). Curr. Alzheimer Res. 15, 259–272.
Ding, Y., Xie, L., Chang, C.Q., Chen, Z.M., Ai, H., 2015. Activation of gamma-aminobutyric acid (A) receptor protects Hippocampus from intense exercise-induced synapses damage and apoptosis in rats. Chin Med J (Engl) 128, 2330–2339.
Disorbo, A., Wilson, G.N., Bacik, S., Hoxha, Z., Biada, J.M., Mickley, G.A., 2009. Time-dependent retrograde amnesic effects of muscimol on conditioned taste aversion extinction. Pharmacol. Biochem. Behav. 92, 319–326.
Dringenberg, H.C., 2000. Alzheimer's disease: more than a 'cholinergic disorder' - evidence that cholinergic-monoaminergic interactions contribute to EEG slowing and dementia. Behav. Brain Res. 115, 235–249.
Ekimova, I.V., 2013. Somnogenic effect of exogenous heat shock protein 70 kDa is mediated by GABA(A) receptors in the preoptic area of the hypothalamus. Dokl. Biol. Sci. 449, 89–92.
Engstrom, F.L., Woodbury, D.M., 1988. Seizure susceptibility in DBA and C57 mice: the effects of various convulsants. Epilepsia 29, 389–395.
Fu, Y., Chen, Y., Li, L., Wang, Y., Kong, X., Wang, J., 2017. Food restriction affects Y-maze spatial recognition memory in developing mice. Int. J. Dev. Neurosci. 60, 8–15.
Fu, Y., Cui, J., Ma, Y., 2011. Differential effects of aging on EEG after baclofen administration. Sci. China Life Sci. 54, 459–465.
Fu, Y., Guo, L., Zhang, J., Chen, Y., Wang, X., Zeng, T., Tian, S., Ma, Y., 2008. Differential effects of ageing on the EEG during pentobarbital and ketamine anaesthesia. Eur. J. Anaesthesiol. 25, 826–833.
Ganguly, R., Guha, D., 2008. Alteration of brain monoamines & EEG wave pattern in rat model of Alzheimer's disease & protection by Moringa oleifera. Indian J. Med. Res. 128, 744–751.
Gibbs, M.E., Johnston, G.A., 2005. Opposing roles for GABAA and GABAC receptors in short-term memory formation in young chicks. Neuroscience 131, 567–576.
Graf, R.A., Kater, S.B., 1998. Inhibitory neuronal activity can compensate for adverse effects of beta-amyloid in hippocampal neurons. Brain Res. 786, 115–121.
Gurevicius, K., Lipponen, A., Tanila, H., 2013. Increased cortical and thalamic excitability in freely moving APPswe/PS1dE9 mice modeling epileptic activity associated with Alzheimer's disease. Cerebr. Cortex 23, 1148–1158.
Hidisoglu, E., Kantar-Gok, D., Er, H., Acun, A.D., Yargicoglu, P., 2018. Alterations in spontaneous delta and gamma activity might provide clues to detect changes induced by amyloid-beta administration. Eur. J. Neurosci. 47, 1013–1023.
Huang, S.H., Duke, R.K., Chebib, M., Sasaki, K., Wada, K., Johnston, G.A., 2003. Bilobalide, a sesquiterpene trilactone from Ginkgo biloba, is an antagonist at recombinant alpha1beta2gamma2L GABA(A) receptors. Eur. J. Pharmacol. 464, 1–8.
Johnston, G.A., 2013. Advantages of an antagonist: bicuculline and other GABA antagonists. Br. J. Pharmacol. 169, 328–336.
Johnston, G.A., 2014. Muscimol as an ionotropic GABA receptor agonist. Neurochem. Res. 39, 1942–1947.

Johnston, G.A.R., 1991. GABAA antagonists. Semin. Neurosci. 3, 205–210.
Jyoti, A., Plano, A., Riedel, G., Platt, B., 2010. EEG, activity, and sleep architecture in a transgenic AbetaPPswe/PSEN1A246E Alzheimer's disease mouse. J. Alzheimer's Dis. 22, 873–887.
Kleschevnikov, A.M., Belichenko, P.V., Villar, A.J., Epstein, C.J., Malenka, R.C., Mobley, W.C., 2004. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J. Neurosci. 24, 8153–8160.
Koss, D.J., Robinson, L., Drever, B.D., Plucinska, K., Stoppelkamp, S., Veselcic, P., Riedel, G., Platt, B., 2016. Mutant Tau knock-in mice display frontotemporal dementia relevant behaviour and histopathology. Neurobiol. Dis. 91, 105–123.
Lee, B.Y., Ban, J.Y., Seong, Y.H., 2005. Chronic stimulation of GABAA receptor with muscimol reduces amyloid beta protein (25-35)-induced neurotoxicity in cultured rat cortical cells. Neurosci. Res. 52, 347–356.
Lei, M., Xu, H., Li, Z., Wang, Z., O'Malley, T.T., Zhang, D., Walsh, D.M., Xu, P., Selkoe, D.J., Li, S., 2016. Soluble Abeta oligomers impair hippocampal LTP by disrupting glutamatergic/GABAergic balance. Neurobiol. Dis. 85, 111–121.
Leon-Espinosa, G., DeFelipe, J., Munoz, A., 2012. Effects of amyloid-beta plaque proximity on the axon initial segment of pyramidal cells. J. Alzheimer's Dis. 29, 841–852.
Leventhal, A.G., Wang, Y., Pu, M., Zhou, Y., Ma, Y., 2003. GABA and its agonists improved visual cortical function in senescent monkeys. Science 300, 812–815.
Li, Y., Sun, H., Chen, Z., Xu, H., Bu, G., Zheng, H., 2016. Implications of GABAergic neurotransmission in alzheimer's disease. Front. Aging Neurosci. 8, 31.
Liu, T., Bai, W., Yi, H., Tan, T., Wei, J., Wang, J., Tian, X., 2014. Functional connectivity in a rat model of Alzheimer's disease during a working memory task. Curr. Alzheimer Res. 11, 981–991.
Lucanic, M., Lithgow, G.J., Alavez, S., 2013. Pharmacological lifespan extension of invertebrates. Ageing Res. Rev. 12, 445–458.
Maggi, A., Enna, S.J., 1979. Characteristics of muscimol accumulation in mouse brain after systemic administration. Neuropharmacology 18, 361–366.
Matsuyama, S., Taniguchi, T., Kadoyama, K., Matsumoto, A., 2008. Long-term potentiation-like facilitation through GABAA receptor blockade in the mouse dentate gyrus in vivo. Neuroreport 19, 1809–1813.
McQuail, J.A., Frazier, C.J., Bizon, J.L., 2015. Molecular aspects of age-related cognitive decline: the role of GABA signaling. Trends Mol. Med. 21, 450–460.
Michelot, D., Melendez-Howell, L.M., 2003. Amanita muscaria: chemistry, biology, toxicology, and ethnomycology. Mycol. Res. 107, 131–146.
Nava-Mesa, M.O., Jimenez-Diaz, L., Yajeya, J., Navarro-Lopez, J.D., 2014. GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic dysfunction in early stages of Alzheimer's disease. Front. Cell. Neurosci. 8, 167.
Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.Q., Kreitzer, A., Finkbeiner, S., Noebels, J.L., Mucke, L., 2007. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55, 697–711.
Palop, J.J., Mucke, L., 2009. Epilepsy and cognitive impairments in Alzheimer disease. Arch. Neurol. 66, 435–440.
Palop, J.J., Mucke, L., 2010. Synaptic depression and aberrant excitatory network activity in Alzheimer's disease: two faces of the same coin? NeuroMolecular Med. 12, 48–55.
Palop, J.J., Mucke, L., 2016. Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat. Rev. Neurosci. 17, 777–792.
Papazoglou, A., Soos, J., Lundt, A., Wormuth, C., Ginde, V.R., Muller, R., Henseler, C., Broich, K., Xie, K., Ehninger, D., Haenisch, B., Weiergraber, M., 2016. Gender-specific hippocampal dysrhythmia and aberrant hippocampal and cortical excitability in the APPswePS1dE9 model of alzheimer's disease. Neural Plast. 2016, 7167358.
Papazoglou, A., Soos, J., Lundt, A., Wormuth, C., Ginde, V.R., Muller, R., Henseler, C., Broich, K., Xie, K., Haenisch, B., Ehninger, D., Weiergraber, M., 2017. Motor cortex theta and gamma architecture in young adult APPswePS1dE9 alzheimer mice. PLoS One 12, e0169654.
Pilipenko, V., Narbute, K., Beitnere, U., Rumaks, J., Pupure, J., Jansone, B., Klusa, V., 2018. Very low doses of muscimol and baclofen ameliorate cognitive deficits and regulate protein expression in the brain of a rat model of streptozocin-induced Alzheimer's disease. Eur. J. Pharmacol. 818, 381–399.
Platt, B., Drever, B., Koss, D., Stoppelkamp, S., Jyoti, A., Plano, A., Utan, A., Merrick, G., Ryan, D., Melis, V., Wan, H., Mingarelli, M., Porcu, E., Scrocchi, L., Welch, A., Riedel, G., 2011. Abnormal cognition, sleep, EEG and brain metabolism in a novel knock-in Alzheimer mouse, PLB1. PLoS One 6, e27068.
Povysheva, N.V., Johnson, J.W., 2016. Effects of memantine on the excitation-inhibition balance in prefrontal cortex. Neurobiol. Dis. 96, 75–83.
Rissman, R.A., Mobley, W.C., 2011. Implications for treatment: GABAA receptors in aging, Down syndrome and Alzheimer's disease. J. Neurochem. 117, 613–622.
Rozycka, A., Liguz-Lecznar, M., 2017. The space where aging acts: focus on the GABAergic synapse. Aging Cell 16, 634–643.
Schmolesky, M.T., Wang, Y., Pu, M., Leventhal, A.G., 2000. Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. Nat. Neurosci. 3, 384–390.
Schneider, F., Baldauf, K., Wetzel, W., Reymann, K.G., 2014. Behavioral and EEG changes in male 5xFAD mice. Physiol. Behav. 135, 25–33.
Verret, L., Mann, E.O., Hang, G.B., Barth, A.M., Cobos, I., Ho, K., Devidze, N., Masliah, E., Kreitzer, A.C., Mody, I., Mucke, L., Palop, J.J., 2012. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell 149, 708–721.
Wang, J., Ikonen, S., Gurevicius, K., van Groen, T., Tanila, H., 2002. Alteration of cortical EEG in mice carrying mutated human APP transgene. Brain Res. 943, 181–190.
Wang, S., Cui, Y., Wang, C., Xie, W., Ma, L., Zhu, J., Zhang, Y., Dang, R., Wang, D., Wu, Y., Wu, Q., 2015. Protective effects of dietary supplementation with a combination of nutrients in a transgenic mouse model of alzheimer's disease. PLoS One 10, e0143135.
Wu, Z., Guo, Z., Gearing, M., Chen, G., 2014. Tonic inhibition in dentate gyrus impairs long-term potentiation and memory in an Alzheimer's [corrected] disease model. Nat. Commun. 5, 4159.
Yajima, Y., Narita, M., Takahashi-Nakano, Y., Misawa, M., Nagase, H., Mizoguchi, H., Tseng, L.F., Suzuki, T., 2000. Effects of differential modulation of mu-, delta- and kappa-opioid systems on bicuculline-induced convulsions in the mouse. Brain Res. 862, 120–126.
Zhang, X., Zhong, W., Brankack, J., Weyer, S.W., Muller, U.C., Tort, A.B., Draguhn, A., 2016. Impaired theta-gamma coupling in APP-deficient mice. Sci. Rep. 6, 21948.